

(SEQ ID NO: 30)		Met	Ser	Lys	Gly	Glu	
	<u>SacII</u>						
oGM1:	5'- GCGCTACCGcggGCCACC	ATG	AGC	AAG	GGC	GAG	3'
(SEQ ID NO: 29)							
pEGFP-CIdKS:5'	GCGCTACCGGT <u>CGCCACC</u>	ATG	GTG	AGC	AAG	GGC	GAG 3'
	<u>AgeI</u>						
(SEQ ID NO: 31)		Val					

(SEQ ID NO: 34)			Ile	Lys	Ala	Asn	Phe	Lys	
oGM2:	5'	GC	ATC	AAG	Gcc	AAC	TTC	AAG	3'
(SEQ ID NO: 33)									
pEGFP-CIdKS:	5'	GC	ATC	AAG	GTG	AAC	TTC	AAG	3'
(SEQ ID NO: 35)									
(SEQ ID NO: 36)				Val					

-Replacement of a 231 Leu codon by a Histidine codon normally present in “wild-type” GFP (Prasher, D.C., Eckenrode, V.K., Ward, W.W., Prendergast, F.G., and Cormier, M.J., Primary structure of the *Aequorea victoria* green-fluorescent protein. *Gene* 111 (1992) 229-233.)

	Ile	Thr	His	Gly	Met	
(SEQ ID NO: 38)						
oGM3: 5'	GG ATC	ACT	CaC	GGC	ATG GA	3'
(SEQ ID NO: 37)						
pEGFP-C1dKS: 5'	GG ATC	ACT	CTC	GGC	ATG GA	3'
(SEQ ID NO: 39)						

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	Ile	Thr	His	Gly	Met
(SEQ ID NO: 38)					
oGM3:	5'	GG ATC	ACT	CaC	GGC
(SEQ ID NO: 37)					
pEGFP-C1dKS:	5'	GG ATC	ACT	CTC	GGC
(SEQ ID NO: 39)					
(SEQ ID NO: 40)			Leu		

Please replace paragraph 79 of the specification, on pages 18-20, with the following paragraph:

Q4

[079] Four PCRs (Polymerase Chain Reaction) done on a vector comprising the aequorin (Aeq) coding phase makes it possible to amplify the A, B, C, and D fragments with, respectively, the primers oAE5A and oAE3A, oAE5B and oAE3B, oAE5C and oAE3C, oAE5D and oAE3D. The overlapping regions are used to assemble the different parts during successive PCRs (Ho, S.N., Hunt, H.D., Horton, R.M., Pullen, J.K., and Pease, L.R. Site-directed mutagenesis by overlap extension using the polymerase chain reaction Gene 77 (1989) 51-49.) An A+B fragment is amplified starting with a mixture of A and B fragments, and primers oAE5A and oAE3B. Similarly, a C+D fragment is amplified with a mixture of C and D fragments, using the primers oAESA and oAE3D. Finally, the complete coding phase, A+B+C+D is developed with the primers oAE5A and oAE3D.

- Each oligonucleotide comprises one or several mismatches that are identified below in lower case. The "wild" sequence is represented opposite, in upper case. The primer oAE5A suppresses the original initiation translation code (ATG) and introduces a Bg/II site. The primer oAE3D introduces an XhoI site just behind the translation

terminal codon (TAA). The final PCR product, digested with the Bg/III and XhoI enzymes, is cloned in the Bg/III-Sa/I sites of the pEGFP-C1mut plasmid in such a way that the Valine codon (GTC), the first codon of aequorin, is the same reading phase as the GFP (see figure). The other primers introduce "silent" mutations that do not change the protein sequence but modify six codons in the jellyfish, *Aequoria Victoria*, to improve their expression in mammals (Wada, K-N., Aota, S.-I., Tshuchiya, R., Ishibashi, F., Gojobori, T., and Ikemura, T. Codon usage tabulated from the GenBank genetic sequence data. Nucleic Acids Res. 18 suppl. (1990) 2367-2411). The completeness of the entire sequence was verified by sequencing,

oAE5A CCATG

5' AGCTTCagatct GTC AAA CTT ACA TCA GAC TTC GAC AAC CCA AGA TGG ATT GGA CGA
3' TCGAAGTctaca CAG TTT GAA TGT AGT CTG AAG CTG TTG GGT TCT ACC TAA CCT GCT

BGIII

CAC AAG CAT ATG TTC AAT TTC CTT GAT GTC AAC CAC AAT GGA AAA ATC TCT CTT GAC GAG
GTG TTC GTA TAC AAG TTA AAG GAA CTA CAG TTG GTG TTA CCT TTT TAG AGA GAA CTG CTC
ATG GTC TAC AAG GCA TCT GAT ATT GTC ATC AAT AAC CTT GGA GCA ACA CCT GAG CAA GCC
TAC CAG ATG TTC CGT AGA CTA TAA CAG TAG TTA TTG GAA CCT CGT TGT GGA CTC GTT CGG

oAE5B A

AAA CGA CAC AAA GAT GCT GTG GAA GCC TTC TTC GGA GGA GCT GGA ATG AAA TAT GGT GTG
TTT GCT GTG TTT CTA CGA CAC CTT CGG AAG AAG CCT CCT CGA CCT TAC TTT ATA CCA CAC

T oAE3A

GAA ACT GAT TGG CCT GCA TAT ATT GAA GGA TGG AAA AAA TTG GCT ACT GAT GAA TTG GAG
CTT TGA CTA ACC GGA CGT ATA TAA CTT CCT ACC TTT TTT AAC CGA TGA CTA CTT AAC CTC

oAE5C G T A

AAA TAC GCC AAA AAC GAA CCA ACC CTC ATC CGC ATC TGG GGT GAT GCT TTG TTT GAT ATC

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TTT ATG CGG TTT TTG CTT GGT TGg GAG TAG GCg Tag ACC CCA CTA CGA AAC AAA CTA TAG

C A T oAE3B

GTT GAC AAA GAT CAA AAT GGA GCT ATT ACA CTG GAT GAA TGG AAA GCA TAC ACC AAA GCT

CAA CTG TTT CTA GTT TTA CCT CGA TAA TGT GAC CTA CTT ACC TTT CGT ATG TGG TTT CGA

GCT GGT ATC ATC CAA TCA TCA GAA GAT TGC GAG GAA ACA TTC AGA GTG TGC GAT ATT GAT

CGA CCA TAG TAG GTT AGT AGT CTT CTA ACG CTC CTT TGT AAG TCT CAC ACG CTA TAA CTA

oAE5D A T A

GAA AGT GGA CAA CTC GAT GTT GAT GAG ATG ACA AGA CAg CAT cTg GGA TTT TGG TAC ACC

CTT TCA CCT GTT GAG CTA CAA CTA CTC TAC TGT TCT GTc GTA gAc CCT AAA ACC ATG TGG

T A T oAE3C

XhoI

ATG GAT CCT GCT TGC GAA AAG CTC TAC GGT GGA GCT GTC CCC TAA TCTcGAGGATCTTT 3'

TAC CTA GGA CGA ACG CTT TTC GAG ATG CCA CCT CGA CAG GGG ATT AGAgCTCCTAGAAA 5'

T oAE3D

(SEQ ID NO: 41)

Please replace paragraph 80, bridging pages 20 and 21 of the specification, with the following paragraph:

[080] In the pEGFPmur-Aeq plasmid, a sequence of five amino acids exists between the coding phases of the GFP and aequorin. Observations led to the lengthening of this region by intercalating a sequence in the *Bsp*EL site. Two complementary oligonucleotides coding for a sequence of nine amino acids give the composition a good deal of flexibility, owing to the abundance of Glycine and Serine. After insertion, the *Bsp*EI site is preserved on only one side although new intercalated sequences may be added successively. At each stage, the orientation is controlled by

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the BspEI enzyme. Two copies of this sequence are needed to restore the normal fluorescence of GFP, but the energy transfer between aequorin and GFP is optimal with five copies. The entire intercalated sequence of pGCA plasmid (5 x 9 aa + the five initial amino acids = 50 aa) was verified by sequencing:

Lys Ser Gly Leu Arg Ser Val (SEQ ID NO: 43)
 5' AAG TCC GGA CTC AGA TCT GTC 3' (SEQ ID NO: 42)
 3' TTC AGG CCT GAG TCT AGA CAG 5' (SEQ ID NO: 44)

GFP BspEI BG111 Aeq

↓

5' AAG T GC GGA CTC AGA TCT GTC 3' (SEQ ID NO: 45)
 3' TTC AGG CC T GAG TCT AGA CAG 5' (SEQ ID NO: 44)

+

Gly Gly Ser Gly Ser Gly Gly Gln Ser (SEQ ID NO: 47)
 5' CC GGC GGG AGC GGA TCC GGC GGC CAG T 3' (SEQ ID NO: 46)
 3' G CCC TCG CCT AGG CCG CCG GTC AGG CC 5' (SEQ ID NO: 48)
 BamHI BspEI

IN THE CLAIMS:

Please cancel claims 4-8, 10-14, 20-25, 28-31, and 34-37

Please amend the claims as follows:

1. (Amended) A method of screening *in vivo* for a change in a physical, chemical, biochemical, or biological, condition, the method comprising the steps of:

a) administering to a mammal a composition comprising a bioluminescent system;

b) detecting whether light is produced; and

Q5

Q6

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